

The immunopathogenesis of multiple sclerosis

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Abstract—Multiple sclerosis (MS) is a T cell-mediated autoimmune disease that is triggered by unknown exogenous agents in subjects with a specific genetic background. Genes of the major histocompatibility complex class II region are the only ones that have been consistently associated with the disease. However, susceptibility is probably mediated by a heterogeneous array of genes, which demonstrate epistatic interactions. Furthermore, an infectious etiology of MS has been suggested, and it is likely that infectious agents shape the immune response against self-antigens. Composition of plaques, response to therapy, and data from animal models indicate that MS is mediated by myelin-specific CD4 T cells that, upon activation, invade the central nervous system and initiate the disease. Different patterns of tissue damage have been shown in active MS lesions, suggesting that the mechanisms of injury are probably distinct in different subgroups of patients. Heterogeneity in clinical characteristics, magnetic resonance imaging, and response to therapies support this notion. The experience gained during several pharmacological studies has improved our understanding of the pathogenesis of MS. New tools, such as gene expression profiling with cDNA microarrays and proteomics, together with advancements in imaging techniques may help us to identify susceptibility genes and disease markers, which may enable us to design more effective therapies and to tailor them according to different disease forms or stages.

Key words: *autoimmune diseases, experimental allergic encephalomyelitis, multiple sclerosis, pathogenesis, therapy.*

INTRODUCTION

Multiple sclerosis (MS) is the most frequent inflammatory demyelinating disease of the central nervous system (CNS) in Northern Europeans and North Americans. It affects mostly young and middle-aged adults leading to substantial disability in more than 50 percent of patients. Its etiology remains unknown, but the composition of plaques, immunogenetic background, response to immunomodulatory and -suppressive therapy, and data from animal models support that MS is an autoimmune disease mediated by myelin-specific CD4 T cells (1,2). Results from a phase II clinical trial with an altered peptide ligand (APL) based on myelin basic protein (MBP) (83–99), which inadvertently exacerbated the disease in some patients, provided the most direct evidence for a pathogenetic role of myelin-specific T cells (3). Heterogeneity in the clinical course, magnetic resonance imaging (MRI), and pathological patterns (4) hinder immunopathogenetic studies. In light of this variability and the lack of specific diagnostic or immunologic markers, many of the potential immune mechanisms postulated to be operative in MS have been studied in a well-defined animal model, experimental allergic encephalomyelitis (EAE). EAE is an acute or chronic relapsing experimental demyelinating disease that is characterized by focal areas of inflammation and demyelination throughout the CNS. It is induced in susceptible animal strains by the injection of myelin or

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myelin components in appropriate adjuvants and is mediated by encephalitogenic T cells (5). Several EAE studies attempted to characterize the specificity, T cell receptor (TCR) expression, major histocompatibility complex (MHC), (Human leukocyte antigen (HLA) in humans) restriction, and functional profile of myelin-reactive T cells. It has recently been shown that transgenic recombinase-deficient (Rag-/-) mice, expressing HLA-DR2 and a human MBP (84–102)-specific TCR, develop spontaneous disease (2). This important work shows that transgenic T cells specific for HLA-DR2-bound MBP (84–102) peptide are sufficient and necessary for the development of disease.

EAE studies greatly contributed to the understanding of the immunopathology of MS; however, controversy still exists as to the relevance of observations in EAE for the human disease. EAE and human studies have also demonstrated a pathogenetic role of autoreactive antibodies and B cells (6), dysregulation of proinflammatory and anti-inflammatory cytokines (7,8), hyperactive Th1 (T helper 1)-mediated immune responses (9), disturbance in costimulatory pathway and apoptosis (10), and reduction in suppressor cell activity.

While the evidence from these studies favors an immunopathogenesis of MS, a recent study has shown that the mechanisms and target of demyelination may be fundamentally different in distinct subgroups or stages of the disease. Heterogeneity in clinical characteristics, MRI, pathology, MR spectroscopy, and response to immunomodulatory therapies support this notion (4). A better understanding of the different pathomechanisms will help us to design more effective therapies and to tailor them according to different disease forms or stages.

POTENTIAL CAUSES OF MS

Genetic Factors

MS has been suggested to be a T cell-mediated autoimmune disease triggered by unknown exogenous agents, such as viruses or bacteria, in subjects with a specific genetic background. Evidence for the contribution of genetic factors to the pathogenesis of MS stems from family and twin studies (11,12). To date, population studies have demonstrated an association in Caucasian MS patients with the class II MHC alleles DRB1*1501, DRB5*0101, and DQB1*0602. These alleles are all con-

tained in the DR2 haplotype, the only one consistently associated with the disease.

For many other candidate genes, an association with MS has not been generally confirmed, probably because genetic analyses are often conducted on poorly stratified and too small populations. Genotypic and phenotypic analyses are now showing that susceptibility is probably mediated by a heterogeneous array of genes, which demonstrate epistatic interaction. In the latter, the genotype at one locus affects the phenotypic expression of the genotype at another locus (13). Linkage with genetic loci was compared for 23 published autoimmune or immune-mediated diseases after genome-wide scans had been performed. The majority of the human positive linkages map nonrandomly into 18 distinct clusters, supporting the hypothesis that, in some cases, clinically distinct autoimmune diseases may be controlled by a common set of susceptibility genes (14). Furthermore, whereas MS patients may have the same susceptibility genes as other patients suffering from different autoimmune diseases, tissue specific genetic factors probably determine which organ is affected in the disease.

Computational genomic sequence comparison between various species can identify plausible regulatory elements, which besides coding sequences might play an important role in autoimmunity (15). Future studies on the genetic influence on MS will have to resolve the question of disease heterogeneity (16).

Exogenous Agents and Molecular Mimicry

An infectious etiology of MS has been indicated by epidemiological studies as well as by similarities to infectious demyelinating diseases. However, infectious agents more likely shape the immune response against self-antigens and may induce disease under special circumstances, rather than implicating a single virus in the case of MS (17). Epidemiological studies have correlated viral infections with exacerbation of MS and have shown that disease prevalence increases with latitude. Migration before puberty from low-prevalence areas to high-prevalence areas results in a higher risk to develop disease (18). A role of infectious agents is further supported by the analysis of MS epidemics: MS was absent from the Faroe Islands (located in the North Atlantic) until World War II when first cases were described and linked to the arrival of the British troops (19).

Viral demyelinating diseases provide examples on how a viral infection may cause demyelination. In JC

virus-induced progressive multifocal leukoencephalopathy (PML), demyelination is caused by a viral infection and direct damage of oligodendrocytes (5). A recent neuropathological analysis of MS lesions has shown a demyelination pattern that appears to be induced primarily by a functional disturbance of oligodendrocytes. The authors hypothesize that it might be the result of infection with an unknown virus or damage mediated by an unknown toxin (4).

In subacute sclerosing panencephalitis (SSPE), virus-infected oligodendrocytes are subject to immune-mediated damage. In postinfectious demyelinating encephalomyelitis, erupting 10 to 40 days following an infection with measles, varicella or vaccinia virus, demyelination is most likely caused by a virus-induced immune response against myelin (20).

As another example, human T cell lymphotropic virus (HTLV)-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) may mimic chronic progressive MS (CPMS), causing progressive myelopathy with atrophy of the spinal cord in 1 to 5 percent of infected individuals. A number of differences can distinguish the TSP and CPMS: TSP shows HTLV-I-specific antibodies, proviral genome in infected cells, and a less-marked demyelination, which is accompanied by a more prominent axonal loss (21). HTLV-I-specific, CD8+, HLA-class I-restricted cytotoxic T lymphocytes have been found at high frequencies in blood, in cerebrospinal fluid (CSF), and in biopsy specimen, providing evidence for the role that immune responses may play in the pathogenesis of HTLV-I-associated neurologic disease (5,22).

An association between HHV-6, a beta herpesvirus with a seroprevalence of 72 to 100 percent in healthy adults worldwide, and MS has been suggested by the demonstration of viral antigen in oligodendrocytes of MS white matter lesion but not in control brain (23). Furthermore, MS patients have been shown recently to have an increased lymphoproliferative response to HHV-6A lysate (24) and elevated antibody titer to HSV-6 antigens in serum and CSF compared with unaffected brains (25). Over the years, several reports have demonstrated increased virus-specific proliferative response in MS patients compared with controls. One must use caution interpreting these data, and additional molecular, serological, and cellular immune response studies are necessary to clarify the role of HHV-6 in MS. Similarly, to what extent CSF oligoclonal IgG bands include antibodies

against Chlamydia antigens still appears to be controversial (26).

Besides a direct role in CNS damage during demyelinating diseases, infectious agents may shape the immune response against self-antigens and may induce disease under special circumstances: MBP-specific T cells can be found in the CSF during postmeasles encephalomyelitis, rubella panencephalitis, and chronic CNS Lyme disease (27,28). Target cells might be damaged as innocent bystanders by the ongoing immune process. Alternatively, infectious agents may trigger an autoimmune response by the infection of target tissues (e.g., oligodendrocytes) via molecular mimicry.

The latter involves reactivity of T and B cells with either peptides or antigenic determinants shared by infectious agents and myelin antigens. A microbial or viral peptide with a certain degree of homology to a self-peptide can stimulate pathogenic self-reactive specific T cells to cause an autoimmune disease. Autoreactive T cells are part of the normal mature immune system. A variety of self-antigens, including MBP and proteolipid protein (PLP), is expressed in thymic epithelial cells. If a T cell recognizes a self-antigen at intermediate levels of affinity in the thymic environment, it will not be deleted; i.e., incomplete clonal deletion will occur and result in the "escape" of autoreactive clones into the peripheral immune repertoire (29).

Autoimmune T cells may be activated by cross-reactive foreign antigens, cross the blood-brain barrier (BBB), infiltrate the CNS, and mediate pathological and clinical damage (30). Complete sequence homology between self-peptide and foreign peptide is not required for molecular mimicry. Single amino acid substitutions in each position of the sequence may be tolerated, cause a reduction or abolition in the response, or generate a superagonist peptide that can be even more potent stimulator of T cell clone functions (31). "Pockets" in the MHC peptide binding groove preferentially "anchor" amino acids with certain chemical properties in specific positions of the antigenic peptides. Many viruses, including Epstein-Barr virus and HHV-6, have been shown to have regions of sequences containing binding motifs for HLA-DR2, and many HLA-DR2-bound microbial peptides can stimulate MBP-reactive T cell clones by cross-reactivity (32). Molecular mimicry is therefore influenced by HLA genes, and individuals bearing the susceptibility-associated HLA alleles may be more prone to pathogen-induced autoimmunity (33).

LOCAL IMMUNOLOGIC EVENTS IN MS LESION

Different patterns of demyelination in active MS lesions have been shown, suggesting that the targets (myelin or oligodendrocytes) and mechanisms of injury are probably distinct in different subgroups of MS patients and at different stages of disease development. These different patterns might reflect different pathogenetic mechanisms of demyelination (4).

As indicated by EAE studies, autoimmune inflammatory diseases of the CNS are initiated by brain-specific T lymphocytes that, upon activation by specific antigens, superantigens, or cross-reacting microbial antigens, invade the CNS via the BBB and initiate the disease (34,35). In particular, after an encounter with foreign antigens, T cells undergo clonal expansion and change from naive to effector phenotype up-regulating costimulatory and adhesion proteins: lymphocyte function-associated (LFA) antigen-1 and very late activation (VLA)-4 molecule that facilitate adhesion to the endothelial cells (EC) layer. Alternatively, T cells might recognize antigens presented by EC and subsequently attack the BBB (36–38). In the early lesion, the expression of EC-activation markers and adhesion molecules (including vascular cell adhesion molecule (VCAM)-1, endothelial cell leukocyte adhesion molecule (E-selectin/ELAM)-1, MHC class II antigens, intercellular adhesion molecule (ICAM)-1 and ICAM-2 and urokinase-activator receptor) is enhanced (39). A recent EAE study has shown that, promptly after injection, the freshly stimulated T cells down-regulate their activation markers, up-regulate a set of chemokine receptors, and increase MHC class II molecules on their surface. Upon arrival in the CNS, the T effector cells are reactivated following an encounter of the autoantigen presented by local antigen-presenting cells (34). Activated T cells deliver help to B cells and secrete proinflammatory cytokines such as interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and later on, chemokines that can chemoattract nonspecific immune cells.

Cytokines, chemokines, and their receptors play an important role in MS (40). A significant increase of serum TNF- α and peripheral blood mononuclear cells (PBMC) expression of IL (interleukin)-12 mRNA was found to precede clinical relapses in patients with relapsing-remitting MS (RRMS). IL-12, produced by antigen-presenting cells, is necessary for developing Th1 response, and IL-12 knockout mice are completely resis-

tant to EAE (41). Chemokines seem to be expressed in the brain secondarily to the initial phase of cell infiltration (42). The chemokines, interferon- γ inducible protein (IP)-10, monokine induced by interferon- γ (Mig), and regulated on activation normal T cell expressed and secreted (RANTES), are increased in the CSF of MS patients during relapse (43). A parallel enrichment in chemokine receptor-bearing cells in the intrathecal compartment has been reported by the same authors. Among others, increased levels of macrophage inflammatory protein (MIP)-1 α were also described in MS lesions in macrophages and microglia. Finally, Th2 cytokines (IL-4 and IL-10) together with TGF- β were increased during phases of remission (7,8).

Inflammatory responses, occurring in parallel and involving negative and positive feedback, are directed against the autoantigen, presumably a component of myelin or oligodendrocytes, and result in demyelination that leads to the development of clinical symptoms. Demyelination may occur by cell-mediated cytotoxicity, antibody- and complement-mediated lysis, toxic effects of TNF- α , oxygen radicals, and nitric oxide.

While less prominent than demyelination, loss of axons in MS is well described and is important in determining clinical disability (44,45). Neuropathologic and imaging studies have recently provided evidence for axonal damage even in the early stages of disease. Axonal loss, detectable in areas of normal-appearing white matter, probably is due to Wallerian degeneration of axons transected in the demyelinating lesions (46). During neurologic disorders associated with neuronal damage, 14-3-3 protein increases in the CSF. In a recent study, the detection of 14-3-3 protein in the CSF, at the first neurologic event suggestive of MS, was associated with conversion to a clinically definite disease in a shorter time (47).

Although evidence exists to support an immunologic function for astrocytes and microglia in CNS inflammation (48–50), the specific role of each cell type in the pathogenesis of MS lesion remains a subject of debate (51). Astrocytes and microglia can secrete anti-inflammatory cytokines such as TGF- β and IL-10, which inhibit Th1 responses. In MS, both microglia and astrocytes become activated and express higher levels of MHC class II molecules (52). In a recent study, microglia and/or macrophages appeared to be the predominant antigen presenting cells (APC). In fact, a monoclonal antibody specific for HLA-DR2-MBP (85–99) complex bound

better to microglia and/or macrophages than to astrocytes in the brain of an HLA-DR2 patient (53).

The inflammation of MS subsequently subsides, at least in most cases, and is paralleled by clinical stabilization. Animal data show that most of the inflammatory cells in the MS plaque undergo apoptosis, whereas other authors have suggested that immunoregulatory cells contribute appreciably to the resolution of inflammation. Fas (CD95) and its ligand (FasL, CD95L) are cell-surface molecules that interact to regulate immune response via induction of apoptosis. Resting T cells express low levels of Fas. Following activation via the antigen receptor of the T cells, the expression of Fas increases within hours and the cells undergo apoptosis in response to the FasL present on other activated T cells. FasL expression has been demonstrated on astrocytes and neurons, and it has been suggested that they may form an immunologic brain barrier, limiting cell invasion during the relapse. Some authors have proposed that in MS, there is a failure of activation-induced cell death (AICD) of autoreactive T cells, and they have shown that IFN- β augment AICD of autoreactive cells up-regulating Fas and FasL (10,54,55). Elevated production of soluble CD95 in RRMS patients, compared with healthy controls, might interfere with CD95-mediated apoptosis and thus limit ongoing immune response (56).

CELLULAR AND HUMORAL RESPONSES IN MS PATIENTS

Contribution of B Cells and Autoantibodies

EAE studies have shown that the disease can be transferred by CD4⁺ T cells but not by humoral factors (57–59), strongly supporting the notion that MS is a T cell-mediated disorder. However, both mutually interacting cellular and humoral immune components may contribute to immune-mediated demyelination. The first hint to an important contribution of humoral factor to inflammatory demyelination in EAE came from the observation that sera from animals affected with EAE displayed demyelinating activity *in vitro* (60). The importance of the humoral component is further supported by the observation that myelin oligodendrocyte glycoprotein (MOG)-specific antibodies enhance clinical severity of EAE and dramatically augment demyelination (61). Furthermore, in the common marmoset (*Callithrix jacchus*) EAE model, autoantibodies against

MOG are responsible for the disintegration of myelin sheaths. Many EAE models lack the early demyelination in the lesions, while this model has a prominent MS-like demyelinating component (6). A large percentage of MS patients is positive for antibodies against an immunodominant MBP peptide (85–99) (62), which is also recognized by MBP-specific T cells derived from HLA-DR2 positive patients, suggesting that sustained antibody responses may be driven by T cells. The antibody response against MOG, a surface-exposed myelin component, is best characterized and has been implicated most convincingly in demyelination (6). Elevated antibody titers against a variety of antigens have been described, including myelin components, oligodendrocyte proteins, viruses, cell nuclei, endothelial cells, fatty acids, gangliosides, and axolemma (63).

From a large pathology sample of MS biopsies and autopsies, four different patterns of demyelination were found: one of these (pattern II) was distinguished from the others by a pronounced Ig reactivity associated with degenerating myelin at the active plaque edge and complement C9neo deposition, suggesting an important role of antibodies (4). Recently, oligodendrocyte precursors have been identified as possible targets of the humoral immune response in some MS patients: an immune attack toward these cells with major remyelinating capacity could compromise repair mechanisms in MS (64).

Intrathecal synthesized oligoclonal IgG or “oligoclonal bands” are present in 95 percent of MS patients throughout the disease. These bands are used as a disease marker and are not affected by treatment with IFN- β (65,66). Sequence analysis of the antigen-binding regions showed a high frequency of clonally expanded memory B cells in the CSF of MS patients (67). Variable heavy chain-4- and chain-1-type antibodies were predominant, and the sequences exhibited extensive somatic mutations, which indicate antigen-driven B-cell selection and not of nonspecific bystander activation (63).

None of these findings allows assigning a primary causative role to humoral factors. Moreover, two human monoclonal antibodies, isolated from serum samples and directed against oligodendrocyte surface antigens, promoted significant remyelination in a virus-mediated model of MS (68). Similarly to the dichotomy of cell-mediated response, where damaging and beneficial roles have been observed, CNS-reactive antibodies are not necessarily pathogenic and may help repair and protect the CNS from immune injury.

Cellular Immune Responses to Myelin Antigens in MS

Even though MS has different histopathologic patterns and the mere presence of autoreactive T cells is not sufficient for disease induction, myelin-specific T lymphocytes are an important prerequisite and appear to play a central role (4,69). T cell reactivity against PLP and MBP has been studied in detail, first in EAE and then in MS (70,71). The fine specificity of these populations was carefully analyzed once it was established that injection of the full-length protein was encephalitogenic and immunogenic and that the disease can be transferred with MBP- and PLP-specific T cells. Following a demonstration that, in animal models, encephalitogenic T cell lines (TCLs) can be generated from bulk cultures by repeated *in vitro* stimulations, a similar approach was taken in MS studies.

Early work largely focused on MBP and showed that very similar or identical areas of this protein are immunodominant in EAE and in MS patients, in particular, MBP (83–99) in the context of DR15, DR4, and DR6 (72–74); MBP (111–129) in the context of DR4 (DRB1*0401); and peptides in the C-terminus in the context of DR15 and DR6 alleles (72–79). The peptide MBP (83–99) is immunodominant in the context of several MS-associated DR alleles (73,74,76–79) and is probably the best-studied autoantigen in human T cell-mediated autoimmune diseases. From these studies, it became clear that a preferential binding of certain myelin epitopes to disease-related HLA/MHC class II molecules does exist and that the antigen-presenting molecules control which peptide is immunodominant. This finding provides an important link between immunogenetic background and the myelin-specific immune response.

A recent study provides evidence that both HLA-DR2 (DRB1*1501) and MBP (84–102)-specific T cells are sufficient and necessary for the development of the disease. The authors developed a mouse model in which the MS-associated HLA-DR2b (DRB1*1501) molecule and DR2b-restricted MBP (84–102)-specific TCR chains were expressed as transgenes. EAE could be induced in the animals and, as well, mice developed spontaneous disease (2). To further stress the importance of MBP (84–102), the same authors were able to demonstrate that HLA-DR2b molecules, expressed by microglia in MS lesion, were the antigen-presenting molecules of the MBP (85–99) peptide. This demonstration provides the

compelling evidence that a myelin peptide is likely a target antigen in MS (53).

Interestingly, very similar or identical areas of the MBP molecule are immunodominant in healthy controls. However, MBP-specific T cells are increased in frequency in MS patients. They also express activation markers, which are a prerequisite for the transmigration in CNS tissue and often can be categorized as proinflammatory Th1 cells based on the secretion of IFN- γ and TNF- α/β (9,76,80). This secretion may be relevant to form new lesion and initiate inflammatory events.

Finally, the most direct evidence that T cell responses against MBP (83–99) have encephalitogenic potential in MS comes from the unexpected results of a phase II clinical study testing an APL (see the next section for details) of MBP (83–99). Three patients out of eight developed exacerbation following administration of an APL. In two of them, immunologic studies could link the increased inflammatory activity seen on an MRI and clinical worsening to a strong immune response against both APL and MBP peptide (83–99) (3).

T cell response against PLP has also been studied in detail. Full-length PLP is exclusively expressed in the CNS where it is the most abundant myelin component. Several PLP epitopes are encephalitogenic in different EAE models (71,81–85) and immunodominant in healthy human control subjects in the context of DR15, DR4, and other HLA-DR alleles (86–89). Furthermore, activated PLP-specific T cells are more frequent in the blood of MS patients (76,90).

Numerous other myelin and nonmyelin proteins have more recently gained attention and have shown to be encephalitogenic in animal models and immunogenic in MS and healthy controls. MOG represents less than 0.05 percent of total myelin protein; it has an immunoglobulin-like extracellular domain that is expressed in abundance in the outermost layer of myelin sheaths, which may render it accessible to antibody attacks (6). It has been shown that anti-MOG antibodies were specifically bound to disintegrating myelin around axons in lesion of acute MS (6). In addition, a number of different MOG peptides are encephalitogenic in various animal models and are targets for myelin-specific T cells (61,91–96).

Other myelin proteins have been studied: myelin-associated oligodendrocytic basic protein (MOBP), oligodendrocyte-specific protein (OSP) and myelin-associated glycoprotein (MAG). MOBP and OSP were able to

induce EAE and were immunogenic in humans (97–100). Involvement of MAG has been addressed in a few studies, which demonstrated reactivity to the protein in MS patients and elevated precursor frequencies in the blood and CSF of MS patients (101–103). α B-Crystallin, transaldolase-H (TAL-H) (104), S-100 (105), and 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNPase) have been also studied.

Reactivity against α B-crystallin was demonstrated when myelin obtained from MS brains or normal white matter was separated by high-performance liquid chromatography (HPLC), and short-term TCL were established against the various fractions. The strongest T cell reactivity was directed against a minor protein component, which was later identified as α B-crystallin. This 23-kDa heat-shock protein is expressed in glial cells in MS plaques (106,107). S-100 elicits a CNS inflammatory response without demyelination and without clinical signs (105). CNPase-specific CD4⁺ T cells could be isolated from both MS patients and controls with the use of CNPase peptides that had been chosen based on the presence of MHC binding motifs for DR2a, DR2b, and DR4Dw4 (99,108). A better knowledge of the characteristics of T cell responses will help better understand the phenotype of MS.

THERAPIES

Before specific immunotherapies can be applied both effectively and safely, we need a better understanding of the complexities of the pathogenesis of T cell-mediated disease, i.e., genetic background, environmental triggers, immune reactivity, vulnerability of the target tissue, and pathological and clinical heterogeneity. On the other side, the experience gained during several pharmacological studies improved our understanding of MS immunopathogenesis.

IFN- β is the first drug with demonstrated immunomodulatory properties, which addresses more specifically the known imbalance of the immune system in MS (109). The major mechanisms of action of IFN- β are the modulation of the expression of adhesion molecules and matrix metalloproteinase, resulting in an inhibition of BBB breakdown, the potential shift of the cellular immune response to a Th2 profile, and the inhibition of MHC expression in a proinflammatory environment (109). However, IFN- β can also transiently increase the

number of IFN- γ -secreting cells (110) and the in vitro and in vivo production of IL-12 receptor β_2 chain and chemokine receptor CCR5, two critical markers of Th1 differentiation (111).

The proposed mechanism of action of Copolymer-1 (Cop-1) (Glatiramer-acetate (GA)) is the functional inhibition of myelin antigen-specific T cell clones, such as those responding to PLP, MBP, and MOG. GA blocks antigen presentation but, more importantly, induces a shift from Th1 to Th2 cytokines and GA-specific Th2 cells, which cross-react with myelin components and thus mediate bystander suppression (112–115).

Even if IFN γ and GA, together with corticosteroid, are the mainstay therapies, they are only moderately effective: they have reduced disease exacerbation by 30 percent or delayed disease progression or onset in large phase III trials (116). Given disease heterogeneity, most treatment will have an impact on some of the immunopathogenetic steps but will have little effect on others. Moreover, current treatments are primarily aimed at blocking the autoimmune process. IFN- β 1a is much less effective in slowing disability progression in secondary progressive multiple sclerosis (SPMS) than it is in RRMS (117). Therefore, to cure the advanced stages of the disease, when inflammation might not be the primary driving force of the disease, we need to develop entirely different therapies aimed at repair.

In addition to IFN- β and GA, several attempts have been made to block the action of autoreactive T cells. APLs are peptides with amino acid substitutions in TCR contact positions that cannot elicit a full agonist response but lead to partial activation (partial agonist), inhibit the response to native peptide by TCR antagonism, or induce bystander suppression (118,119). Bystander suppression relies on T cells that are able to cross-react with the native peptide, secrete Th2 and Th3 cytokines (120), and migrate to the inflamed target organ where they are locally reactivated and lead to cytokine secretion (121).

APLs derived from MBP (83–99) and from PLP (139–151) (120,122–125) were successfully used in the treatment of EAE and showed beneficial effects. In light of these promising results, for the first time, a phase II clinical trial was conducted to study the APL of MBP (83–99) CGP77118 (3). As we have previously mentioned, three patients out of eight suffered exacerbation following administration of an APL. Two of the patients' clinical worsening were linked to a strong immune response against both an APL and MBP peptide (83–99).

Although APL-specific T cells had expanded (which is an important prerequisite for “bystander suppression,” the most likely involved mechanism of action), these cells often did not express the therapeutic desired anti-inflammatory phenotype but were Th1 instead. Data from a multicenter phase I trial with the same peptide showed that lower doses tended to skew the cytokine phenotype of APL-specific T cells toward Th2, whereas the high doses preferentially led to Th1 cells.

Another lesson comes from the treatment with a TNF- α receptor-immunoglobulin G1 fusion protein, which was protective in EAE but not effective in a randomized placebo-controlled multicenter study. These results remind us that cytokines are pleiotropic factors and act in a complex network and certain actions of TNF- α may be viewed as proinflammatory, while others are reviewed as anti-inflammatory. Thus, blockage of TNF- α might augment those responses that contribute to MS pathogenesis (126). The administrations of a cytokine that is thought to exert an anti-inflammatory action or the inhibition of a proinflammatory cytokine or its receptor are strategies that are likely going to fail if used as monotherapy. In fact, other pathways in this complex network can compensate for the blocked or enhanced cytokine and, if the targeted factor has a dual role, the treatment might be deleterious.

The combination of treatment principles that interfere with the autoimmune process at multiple levels will likely be beneficial, and research should be oriented toward this goal. However, the safety and the potential interaction must be assessed, since unexpected reaction or lack of effect might occur.

A clinical trial, designed to test possible synergistic effects of GA and type I interferon in EAE, demonstrated the association of the two drugs slightly worsened the disease, even if each compound per se was effective (127). However, this was not confirmed by a later multicenter trial that reported good tolerability and a trend toward efficacy (128).

The inhibitors of phosphodiesterase (PDE)-4 and -3, predominantly expressed in immune cells, have been shown to have the potential to modulate immune response from the Th1 to the Th2 phenotype both in EAE (129,130) and in *in vitro* culture of human CD4⁺ T cell. As TCLs from MS patients have demonstrated a higher susceptibility to the treatment than control TCLs, PDE inhibitors may be used with other therapies to widen the therapeutic window, without inducing a profound immunosuppression (131). Finally, two human monoclonal

antibodies that were directed against oligodendrocyte surface antigens promoted significant remyelination in a virus-mediated model of MS (68).

New tools, such as gene-expression profiling with cDNA microarrays and proteomics, together with advancements in imaging techniques, may help us to improve our knowledge of susceptibility genes and to identify disease markers so as to design more effective therapies and to tailor them according to different disease forms or stages.

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